Expression of Human IgG Subclasses*

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ABSTRACT

Human immunoglobulin G (IgG) can be divided into four subclasses that are selectively expressed. For instance, carbohydrate antigens preferentially elicit IgG2 antibodies, whereas protein antigens usually elicit IgG1 and IgG3. Elucidating the biological basis of the selective expression of these IgG subclasses is important to our understanding immunodeficiencies and B lymphocyte development. To investigate clinical importance of IgG subclass deficiencies, a sensitive and specific assay has been developed for IgG subclasses using particle concentration fluorescence immunoassay. Preliminary clinical studies have already shown that infection-prone individuals often have selective IgG2 subclass deficiency. Normal levels of IgG2, however, do not rule out an immunodeficiency in the infection-prone individuals because some individuals have normal levels of IgG subclasses and are poorly responsive to antigens of bacteria.

Based on animal studies, two contrasting models of B cell development have been advanced. One model of B cell development proposes a single lineage and proposes that a B cell can successively switch and produce any IgG subclass. The other model proposes multiple lineages and proposes that a B cell can express only some IgG subclasses. It has been found by us that anti-PC antibodies are mostly IgG2 with some IgG1, and that the V region of IgG1 anti-PC antibody is different from that of IgG2 antibody. Our finding, therefore, suggests that B cells producing anti-PC antibodies are progeny of not one ancestral B cell that has successively switched, but two independent ancestral B cells. Cellular studies using polyclonal activators also suggest that regulatory mechanisms for IgG1 and IgG3 are different from those of IgG2 and IgG4. Taken together, we favor the multilineage model better than the single lineage model of human B cell development.

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Introduction

Immunoglobulins (Ig) consist of heavy (H) and light (L) polypeptide chains held together by non-covalent forces and
disulfide bonds. The variable region of an immunoglobulin, which contains the antigen binding site, is formed by the amino terminal 105–120 amino acids of the heavy and light chains. Tremendous structural diversity of antigen binding sites is achieved by a specialized genetic process for encoding these amino terminal segments. The process involves a presumably random combination of multiple gene segments; V_H, D_H, and J_H for the heavy chain variable regions, and V_L and J_L for the light chain variable regions. Since there are hundreds of V genes and many D and J genes, a very large number of antigen binding sites can be formed. In contrast, the carboxy terminal segments, or constant regions of heavy and light chains, are encoded by only one of nine C_H genes for the heavy chain and one of two C_L genes for the light chain.

Immunoglobulin molecules are categorized into various isotypes based on their heavy chain constant region. In humans, these are IgM, IgD, IgA1, IgA2, IgE, and four IgG subclasses, IgG1, IgG2, IgG3, and IgG4. In mice, the IgG subclasses are IgG1, IgG2a, IgG2b, and IgG3. The constant region of an Ig is responsible for various biologic properties of the molecule, including complement fixation, opsonic properties, placental transfer, and Fc receptor binding.

In view of the biological differences among the IgG subclasses, it is not surprising that the IgG subclasses are selectively expressed (table I). First, the antibody response to different types of antigens seems to favor certain subclasses. For example, many carbohydrate (CHO) antigens preferentially elicit IgG responses restricted to IgG3 in mice and to IgG2 in humans. In contrast, IgG1 dominates the IgG response to many protein antigens in both species. Secondly, acquisition of adult levels of IgG2 in humans is delayed during ontogeny. It is speculated that the delayed maturation of the ability to produce an immune response to CHO antigens is linked to this late maturation of IgG2. Interestingly, the response to CHO antigens is also delayed during ontogeny in mice. Thirdly, expression of certain IgG subclasses is selectively suppressed in certain immunodeficiencies. Patients with ataxia-telangiectasia have selective deficiencies of IgA and of the IgG2 and IgG4 subclasses. CBA/N mice have an X-chromosome-linked immunodeficiency that results in selective deficiency of the IgG3 subclass. Lastly, a subclass specific lymphokine (BSF-1) has been identified in the mouse which preferentially facilitates IgG1 and decreases IgG3 expression following mitogen stimulation. These observations, summarized in table I, strongly suggest a mechanism(s) that selectively regulates expression of the IgG subclasses.

To explain selective IgG subclass expression, two contrasting models of B cell development have been proposed. One model proposes that all B cells
develop in a single lineage and can express all IgG subclasses. In this model, selective expression of IgG subclasses is solely the result of exogenous factors, such as T cells, inducing successive expression of the IgG subclasses according to their order in the genome. A strict extrapolation of this model to human B cells is depicted in figure 1. The alternative model proposes that multiple B cell lineages exist which are restricted in IgG subclass expression and which develop at different times in ontogeny. Because the two models are of fundamental importance in understanding B cell development and regulation, they have been intensely studied in experimental animals. Thus far, neither model can fully explain all of the observations.

Because of the biological and clinical importance of non-random subclass expression, studying this phenomenon in humans was of interest to us. It was felt that human studies might provide a fresh perspective on the models which had been proposed on the basis of experimental animal studies. Our aims were (1) to identify patients with specific subclass deficiencies; (2) to characterize the human IgG subclass responses to several CHO antigens; and (3) to study the cellular basis of IgG subclass expression. An essential pre-requisite to accomplish

![Figure 1](image-url)
these aims was the development of sensitive and specific assays for human IgG subclasses. These assays, and the results of our studies of IgG subclass expression are described.

Development of Sensitive and Specific IgG Subclass Assays

The constant regions of human IgG subclasses have a very high degree of homology, yet have a large degree of allotypic variation. Consequently, ever since the serological definition of IgG subclasses, their study has been hampered by difficulties in measurement. In order to produce specific antisera to IgG subclasses, a variety of animals, including monkeys, has been immunized with human myeloma proteins. Exhaustive immunochemical manipulations have been required to render these antisera specific. Even when a satisfactory antiserum has been prepared for a particular assay, it often lost its specificity when used in a different assay configuration. For example, many of the reagents were limited for use in the relatively insensitive immunodiffusion assay or in the technically difficult hemagglutination assay.

In order to investigate the cellular basis of isotype restriction, extremely sensitive assays were required to measure in vitro IgG subclass production. Thus, sensitive and specific radioimmunoassays (RIA) for the measurement of all four IgG subclasses were developed by us. Our initial RIA's used murine monoclonal antibodies specific for IgG1 and IgG3, and specific, absorbed antisera, raised in monkeys and sheep for IgG2 and IgG4, respectively. The subclass specific antibodies were coated onto the wells of 96-well polyvinylchloride microtiter plates. To these wells, either samples or myeloma protein standards were added and titrated as inhibitors of the binding of an ¹²⁵I-labeled purified myeloma protein of the appropriate subclass. The concentration of the IgG subclasses was calculated by comparing the inhibition produced by standards and samples. The sensitivity was the minimum amount of standard which produced 50 percent inhibition and was generally 50 to 200 ng per ml. Specificity of the reagents was confirmed with a panel of myeloma proteins. The average inhibition curves with a series of myeloma proteins from each subclass are shown in figure 2. However, RIA results were poorly reproducible from plate to plate, presumably because of variation in microtiter plates that affected the efficiency of solid phase antibody coating.

Since it had been established by us that the specificity of some of the subclass-specific reagents was dependent on the assay configuration, it was wished that the reproducibility of our assays would be improved without altering the configuration. Therefore, a new analytical method called particle concentration fluorescent immunoassay (PCFIA), was utilized. In this assay, 0.8 micron latex particles were coated with IgG subclass specific antibodies and placed in specialized microtiter plates (figure 3). Samples or standards were added to the wells as competitive inhibitors of the binding of fluorescein labelled myeloma proteins of the appropriate subclass. Following an incubation period, unbound fluorescent myeloma protein was removed by washing and filtration. The fluorescence bound to the latex particles was inversely related to the amount of that IgG subclass in the sample. This assay achieves high sensitivity by concentrating the latex particles to a very small area by filtration, after which the fluorescence is read. Also because specialized plates with many individual wells are used, a large number of samples can easily be analyzed. While the inhibition configuration similar to that used in our RIA's was generally main-
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CONCENTRATION OF INHIBITOR PROTEIN (ng/ml)

FIGURE 2. Specificity of subclass specific radioimmunoassays. Shown are mean inhibition curves of four IgG1 (●—●), five IgG2 (○—○), three IgG3 (△—△), and three IgG4 (□—□) proteins which include proteins of each light chain type. Bars denote standard deviation.

tained, the PCFIA method is also adaptable to "sandwich" type double antibody assays.

Specificity was confirmed for each of the PCFIA subclass assays with a panel of myeloma proteins. Accuracy of the assay system was confirmed in four ways: (1) For each subclass, the curves produced by several different myeloma standards were shown to be parallel; (2) The sum of the subclass values was close to total IgG values determined by an independent method; (3) The correlation between PCFIA and RIA results was excellent; and (4) Using myeloma standards, correct values were obtained for the WHO reference serum. The major advantage of this system over RIA was the increased precision (CV = 10 percent). However, an additional advantage was the capability of performing numerous (100) determinations in a short period of time (two hours). The sensitivity (0.3 to 3 μg per ml) of these assays was less than that of the RIA's, but was sufficient for all purposes, except measurement of secreted IgG subclasses in some in vitro cultures. These assays are now routinely used to study total IgG subclass concentrations in serum and in affinity purified antibody preparations.

Subclass-Specific Immunodeficiency

In order to study patients with IgG subclass deficiencies, it was first necessary to establish normal ranges in healthy individuals. Using the subclass specific RIA, IgG subclass concentrations were measured in sera from a large number of children and adults and the geometric means and normal ranges
determined for various age groups (±2 S.D. about the mean) (table II). These normal ranges were similar to previously published values. It was also confirmed by us that, in children, acquisition of adult IgG2 levels is delayed, when compared to the other subclasses.

Next, IgG subclass concentrations in sera were prospectively examined from 29 children of different ages with recurrent bacterial infection. Seven of these patients had IgG2 deficiency, defined as a serum concentration which was greater than 3 S.D. below the appropriate age-group mean. Interestingly, only one of these children also was deficient in IgG1 (table III). Clinical improvement was seen in three of four children with IgG2 deficiency who received replacement gamma globulin therapy. Other recent reports have emphasized the possible role of IgG2 deficiency in individuals with recurrent sinopulmonary infections and otitis media. Although these studies used different criteria for IgG2 deficiency (greater than 2 S.D. below the mean) and were largely based on retrospective analysis, it appears that selective IgG2 deficiency is frequent among children with recurrent bacterial infections. These observations suggest that serum concentrations of IgG subclasses should be measured in individuals with recurrent infections.

Previous reports of preferential expression of IgG2 in antibody responses
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TABLE II

IgG Subclass Concentrations (mg/ml) in Healthy Subjects

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>IGG1 (n)* [bounds]</th>
<th>IGG2 (n) [bounds]</th>
<th>IGG3 (n) [bounds]</th>
<th>IGG4 (n) [range]</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5-1</td>
<td>3.7(7)* [1.5-8.7]</td>
<td>0.77(7) [0.26-2.3]</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>6.0(26) [2.3-16.0]</td>
<td>1.1(26) [0.46-2.7]</td>
<td>0.19(10) [0.030-0.95]</td>
<td>0.030(9) [0.009-0.11]</td>
</tr>
<tr>
<td>2</td>
<td>5.4(23) [2.1-14.0]</td>
<td>1.3(23) [0.67-2.4]</td>
<td>0.19(10) [0.051-1.1]</td>
<td>0.02(10) [0.004-0.42]</td>
</tr>
<tr>
<td>3-7</td>
<td>5.5(22) [3.0-10.0]</td>
<td>1.5(22) [0.54-4.5]</td>
<td>0.25(21) [0.58-1.1]</td>
<td>0.21(21) [0.010-0.92]</td>
</tr>
<tr>
<td>8-16</td>
<td>6.9(26) [2.2-14.0]</td>
<td>1.7(26) [0.56-5.3]</td>
<td>0.32(13) [0.095-1.1]</td>
<td>0.36(12) [0.017-1.7]</td>
</tr>
<tr>
<td>&gt;17</td>
<td>6.6(41) [3.6-12.0]</td>
<td>2.6(41) [0.88-7.4]</td>
<td>0.32(19) [0.072-1.4]</td>
<td>0.47(19) [0.048-3.6]</td>
</tr>
</tbody>
</table>

*Values shown are geometric means. The normal bounds for IgG1, IgG2, and IgG3 in brackets were determined by taking the antilog of (mean logarithm ± 2 S.D. of the logarithms). For IgG4, because of extremely wide variation, the normal values in brackets indicate the range. The number in parentheses indicates the number of individuals studied for each group.


Immunoglobulin Concentrations (mg/ml) in IgG2 Deficient Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (Years)</th>
<th>Clinical Features*</th>
<th>IgG</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>19</td>
<td>P, A</td>
<td>9.5</td>
<td>8.7</td>
<td>0.053†</td>
<td>0.14</td>
<td>0.002‡</td>
<td>&lt;0.06‡</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.6)†</td>
<td></td>
<td></td>
<td>(4.0)†</td>
<td>(0.51)</td>
<td>(0.072)</td>
<td>(0.010)</td>
<td>(0.70)</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>S</td>
<td>7.4</td>
<td>7.0</td>
<td>0.098‡</td>
<td>0.087</td>
<td>0.019</td>
<td>0.28</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.2)</td>
<td></td>
<td></td>
<td>(2.7)</td>
<td>(0.49)</td>
<td>(0.051)</td>
<td>(0.005)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>P, P, FTT</td>
<td>4.3‡</td>
<td>4.1</td>
<td>0.053‡</td>
<td>0.12</td>
<td>0.035</td>
<td>&lt;0.06‡</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.3)</td>
<td></td>
<td></td>
<td>(3.0)</td>
<td>(0.32)</td>
<td>(0.058)</td>
<td>(0.010)</td>
<td>(0.33)</td>
</tr>
<tr>
<td>4</td>
<td>1.7</td>
<td>P, P, FTT</td>
<td>6.2</td>
<td>4.7</td>
<td>0.15‡</td>
<td>0.39</td>
<td>0.005‡</td>
<td>0.29</td>
<td>0.61</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.5)</td>
<td></td>
<td></td>
<td>(2.4)</td>
<td>(0.30)</td>
<td>(0.039)</td>
<td>(0.005)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>5</td>
<td>2.3</td>
<td>Hib</td>
<td>9.0</td>
<td>9.3</td>
<td>0.30‡</td>
<td>0.53</td>
<td>0.003‡</td>
<td>2.4</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.2)</td>
<td></td>
<td></td>
<td>(2.7)</td>
<td>(0.49)</td>
<td>(0.051)</td>
<td>(0.005)</td>
<td>(0.14)</td>
</tr>
<tr>
<td>6</td>
<td>7.2‡</td>
<td>P, S, P, FTT</td>
<td>4.1‡</td>
<td>1.5‡</td>
<td>0.23‡</td>
<td>0.51</td>
<td>0.20</td>
<td>1.5</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6.3)</td>
<td></td>
<td></td>
<td>(3.0)</td>
<td>(0.94)</td>
<td>(0.058)</td>
<td>(0.010)</td>
<td>(0.33)</td>
</tr>
<tr>
<td>7</td>
<td>3.2</td>
<td>M</td>
<td>16.00</td>
<td>6.6</td>
<td>0.29‡</td>
<td>0.26</td>
<td>0.46</td>
<td>1.1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(4.4)</td>
<td></td>
<td></td>
<td>(4.4)</td>
<td>(3.0)</td>
<td>(0.54)</td>
<td>(0.058)</td>
<td>(0.010)</td>
</tr>
</tbody>
</table>

*P = pneumonia; S = sinusitis; O = otitis media with drainage; Hib = recurrent invasive Haemophilus disease; M = meningitis; A = chronic polyarthritis; FTT = failure to thrive (<5th percentile).
†Values in parentheses for IgG, IgA, IgM, IgG1, IgG3, and IgG4 are the lower limits of normal as defined in Table 2. Value for IgG2 is 3 SD below the mean for age.
‡Values below normal range.
§Patient also has thrombocytopenia.


to CHO antigens raised the possibility that individuals who responded poorly to these antigens may be deficient in IgG2. Umetsu et al52 have shown that the ability to respond to Haemophilus influenzae type b (Hib), polysaccharide vaccine is impaired in children with IgG2 deficiency. However, patients with Wiskott-
Aldrich syndrome, an X-chromosome linked, inherited disorder associated with numerous immunological abnormalities, including poor responses to CHO antigens and severe recurrent bacterial infections, have normal serum levels of IgG2 (figure 4). Also, serum IgG2 concentrations were normal in 33 children who developed invasive *H. influenzae* disease in spite of immunization with type b polysaccharide vaccine. These children had low type b polysaccharide antibody concentrations in convalescent serum following disease. These studies suggest that deficient anti-CHO-antibody responses may occur in individuals with normal serum concentrations of IgG2. Therefore, evaluation of infection-prone individuals should include measurement of specific anti-CHO antibodies, as well as serum concentrations of IgG subclasses.

**Isotype Restriction of Antibodies**

As mentioned previously, two models of B cell development have been proposed on the basis of animal studies. The single lineage model is supported by the demonstration that a single B cell can express all isotypes under experimental conditions. According to this model,
all IgG subclasses can be expressed successively according to their gene order, with T cells facilitating the successive downstream switching. According to this model, CHO antigens induce expression of IgG3 in mice because the response is T cell independent and thus select the constant region gene (C_3\gamma) nearest to the C_\mu gene.\textsuperscript{50} In humans, however, the 5' \rightarrow 3' CH region gene order is C_\mu, C_\beta, C_\gamma3, C_\gamma1, C_\alpha1, C_\gamma2, C_\gamma4, C_\alpha, C_\alpha2. A proximal (5' end) gene cluster contains C_\gamma3, C_\gamma1, and C_\alpha1 while a distal (3' end) cluster contains the last four genes.\textsuperscript{7} Thus, in humans, the single lineage model would predict that T-independent CHO antigens would preferentially elicit the IgG3 subclass. However, prior observations, although limited, have shown preferential expression of IgG2 by these antigens.\textsuperscript{31,56} Thus, additional studies were performed of the IgG subclass distribution of anti-CHO antibodies to test models of B cell development in humans.

Human antibodies were examined to phosphocholine (PC), the immunodominant antigen in the cell wall CHO of\textit{ Streptococcus pneumoniae}, to group A streptococcal CHO (GAC), and to the polysaccharide capsule of\textit{ Haemophilus influenzae} type b (Hib PS).\textsuperscript{41} As reported by previous investigators,\textsuperscript{4,31} it was found by us that IgG2 is the dominant subclass in naturally occurring antibodies to PC (table IV) and GAC (unpublished data). These results demonstrate the limitation of the linear model of B cell development in humans as it cannot readily explain the preferential expression of the distally located C_\gamma2 gene in these anti-CHO antibody responses.

However, the restriction of anti-CHO antibodies to IgG2 is not absolute. One individual subject produced significant proportions of anti-PC antibodies in the IgG1 subclass (46 percent) and another produced significant amounts of anti-PC in IgG2 (44 percent). In addition, when the IgG subclass composition of anti-Hib PS antibodies in vaccinees was studied, substantial quantities were found of both IgG1 and IgG2 (table IV).\textsuperscript{41} In children, anti-Hib PS antibodies were predominantly IgG1.\textsuperscript{13} Additional studies have also shown that children produce IgG1 antibodies to pneumococcal capsular polysaccharide antigens.\textsuperscript{10} Thus, a successful model of B cell development must explain these exceptions in addition to the apparent IgG2 restriction noted above with respect to antibodies to PC and GAC.

To understand better the complex expression of IgG subclasses, the antigen binding specificity of anti-PC antibodies of IgG1 and IgG2 subclasses were studied. The single lineage model of B cell development suggests that a particular V

\begin{table}[h]
\centering
\caption{IgG Subclass Distribution of Antibodies to PC and Hib Ps in Sera from Healthy Adults}
\begin{tabular}{|l|c|c|c|c|}
\hline
Antibody & n & Percent IgG1\textsuperscript{*} & Percent IgG2 & Percent IgG3 & Percent IgG4 \\
\hline
Anti-PC, healthy adults & 26 & 12 \pm 10 (1-46)\textsuperscript{\dagger} & 83 \pm 13 (48-100) & 6 \pm 10 (1-44) & <1 \\
Anti-Hib PS, immunized adults & 6 & 59 \pm 16 (31-77) & 41 \pm 16 (23-69) & ND & ND \\
\hline
\end{tabular}
\textsuperscript{*To calculate the percentage for anti-PC, total IgG was assumed to be IgG1 + IgG2 + IgG3; for anti-Hib PS, total IgG was assumed to be IgG1 + IgG2. IgG3 and IgG4 anti-Hib PS antibodies have been assumed to be negligible.}
\textsuperscript{\dagger}Mean \pm S.D. (Range)
\textsuperscript{Data are adapted from Scott, et al., 1987 and Shackelford, et al., 1987.)
\end{table}
region may associate with both IgG1 and IgG2 antibodies. However, previous studies in mice have shown that different V regions, or binding specificities, are preferentially associated with either IgG1 or IgG3 antibodies. Our studies of anti-PC antibodies in humans showed that IgG2 anti-PC antibodies expressed V regions which bound PC either when it was conjugated to a protein carrier or when it was a component of the C-carbohydrate (C-CHO) of S. pneumoniae (figure 5). In contrast, IgG1 anti-PC antibodies bound PC only when it was conjugated to a protein. Thus, there are differences in the fine specificity of PC-binding V regions associated with IgG2 and those associated with IgG1. This observation indicates that in humans, as well as in mice, certain V regions preferentially pair with certain IgG subclasses and supports the multiple B cell lineage model.

How selective pairing of V and C_H regions may affect protective immunity was also speculated by us. For example, it was questioned whether IgG2 antibodies differ from IgG1 antibodies in acti-

**Figure 5.** Agarose isoelectric focusing of anti-PC antibody from a single donor. Gels were overlayed with either 125I-PC-BSA (panel A) or 125I-C-CHO (panel B). Lanes marked W are antibodies enriched by affinity chromatography using PC-conjugated Sepharose 4B. Lanes marked G1 and G2 are IgG1 and IgG2 fractions obtained by passing the enriched antibody over columns of monoclonal anti-IgG1 or anti-IgG2 conjugated to Sepharose 4B and eluting with 0.1 M glycine-HCl (pH 2.5). IgG2 anti-PC antibodies bind both PC-BSA and C-CHO. IgG1 anti-PC antibodies bind only PC-BSA.
vating complement-mediated bactericidal activity or protective activity. Briles et al\textsuperscript{3} have shown that murine anti-PC antibodies with three different IgG constant regions were similar in their capacity to protect mice from experimental infection with \textit{S. pneumoniae}. In collaboration with Drs. Winberg and Granoff, it was found by us that the functional capacity of human IgG1 and IgG2 anti-Hib PS antibodies was similar as measured by \textit{in vitro} complement-mediated bactericidal assay and \textit{in vivo} rat protection activity (figure 6).\textsuperscript{55} Thus, differences among Fc mediated biological activities cannot be responsible for the preferential expression of IgG subclasses.

**Cellular Events in IgG Subclass Expression**

To study the cellular mechanisms of IgG subclass expression \textit{in vitro}, IgG subclass expression by human lymphocytes was examined following stimulation with various mitogens.\textsuperscript{37} Because mitogens stimulate large fractions of B cells to proliferate and mature into plasma cells, they can be used to identify a cell subpopulation important for the expression of certain IgG subclasses. Studies in mice have shown that some mitogens preferentially stimulate a subpopulation of B cells, resulting in secretion of only certain IgG subclasses.\textsuperscript{20} Although humans have a large number of

![Figure 6. Passive protection of newborn rats with isolated human IgG1 and IgG2 antibodies to the capsule of \textit{H. influenzae} type b (Hib). The X axis shows the dose of IgG1 or IgG2 anti-capsular antibody injected subcutaneously at time 0. Twenty-four hours later, animals were injected intraperitoneally with 100 colony-forming units of Hib. The Y axis shows the percent of rats with bacteremia 24 hours after bacterial challenge. Numbers in parenthesis show bacteremic rats/rats injected. Adapted from Weinberg et al. J. Immunol. 136:4232, 1986](image-url)
IgG2-bearing B cells among peripheral blood lymphocytes, many mitogens preferentially elicit secretion of human IgG1 and IgG3 subclasses and are poor stimulators of IgG2 and IgG4 (figure 7). Thus, it is possible that IgG1 and IgG3 subclasses are expressed and regulated as a “set” while IgG2 and IgG4 are included in a different “set.”

Several additional observations group the four IgG subclasses similarly into two “sets”. The IgG1 and IgG3 genes are closely linked in one location on chromosome 14 whereas the IgG2 and IgG4 genes are in another, more distal location. Individuals deficient in IgG2, also frequently have a deficiency of IgG4. Finally, when the histology of lymph nodes is examined, germinal centers are found to contain cells bearing IgG1 or IgG3 but not IgG2 or IgG4. Taken together, these observations favor the concept of separate B cell lineages that express the IgG1/IgG3 or IgG2/IgG4 “sets” of subclasses and respond to different antigens and lymphokines. However, to delineate clearly the cellular mechanisms for the preferential expression of IgG subclasses, further studies are needed. In this regard, the study of individuals with selective IgG subclass deficiency may provide important insights.

Summary

Sensitive and specific competitive inhibition assays for human IgG subclasses have been developed by us. Adaptation of PCFIA to IgG subclass analysis has greatly facilitated our studies. These assays have allowed us to establish the prevalence of IgG subclass-specific immunodeficiency, to examine the extent of subclass restriction of antibodies to CHO antigens in humans, and to begin to study cellular mechanisms of IgG subclass expression. It is believed that the regulation of IgG subclasses will be complex, involving B cell subpopulations which are relatively, but not absolutely, precommitted to particular IgG subclasses and, possibly, to particular V regions. Although the mechanisms behind the described observations remain to be elucidated, its elucidation will provide us valuable insights for our clinical management of the common, IgG subclass specific immunodeficiencies.

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